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(71) Applicant: FLORIDA STATE UNIVERSITY [US/US]; Tallahassee, FL 32306 (US).

(72) Inventor: DE KLOET, Siwo, R.; 4355 Cripple Creek Drive, Tallahassee, FL 32308 (US).

(74) Agents: MANSO, Peter, J. et al.; Ruden, Barnett, McClosky, Smith, Schuster & Russell, 200 East Broward Boulevard, P.O. Box 1900, Fort Lauderdale, FL 33302 (US).

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(54) Title: SEX-SPECIFIC DNA PROBE FOR PARROTS, METHODS AND KITS

(57) Abstract

Novel DNA probes for determining the sex of parrots in captive breeding programs and in wild populations are disclosed. The unique DNA probes of the present invention are sex specific, but not parrot-species specific. In addition, the DNA probes of the present invention are reliable, yield results within approximately 24 hours, easy to use, and are not harmful to the parrots being tested.

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SEX-SPECIFIC DNA PROBE FOR PARROTS, METHODS AND KITS

Field of the Invention

The present invention relates to DNA or RNA probes for binding specifically to female DNA of parrots to determine the sex of parrots within a single day and methods and kits.

Background

Parrots is the collective name for approximately 350 species of birds scientifically known as the Psittacidae genus. Parrots include well-known species such as cockatoos, macaws, parakeets and amazones.

Parrots are among the most popular pet birds because of the ability of many species to mimic human speech and to develop strong bonds with their caretakers. However, as a result of habitat loss and the pet bird trade, many species of parrots have become rare or endangered in the wild.

In order to protect populations of wild parrots, many countries have restricted or completely forbidden the exportation of parrots. Moreover, captive breeding has become an important procedure to prevent further depletion of wild populations and to satisfy the demand for parrots in the pet bird market.

For such breeding projects, male and female birds are needed. Therefore, the ability to determine the sex of individual birds is of major importance. Unfortunately, most species of parrots are monomorphic, meaning that there are no recognizable visible or audible differences between male and female birds. More specifically, virtually all juveniles and about 60% of the adults of all avian species are monomorphic.

Presently, there are basically five methods relied upon today to determine the sex of birds. Unfortunately, the methods are not without drawbacks. In one method, the external reproductive organs are physically examined to the extent possible. This method, called "vent-sexing," is applicable for a few bird families where the external reproductive organs are large enough that they can be observed with relative ease in the cloaca. This method is, in practice, only applicable to waterfowl, such as ducks, geese, and swans, and gallinaceous birds, such as chickens, where the method can be

applied at an early age. However, the difficulty in handling, as well as the difficulties in observing the external reproductive organs, make "vent-sexing" unsuitable for use with parrots, as well as other birds in which the external reproductive organs cannot be observed.

A second method, which is an invasive method, involves the examination of internal reproductive organs. According to this method, a bird must be placed under general anaesthesia and an incision made so that the internal reproductive organs can be observed with an endoscope. Although this is the most often used procedure, it causes great trauma to the bird and often results in infection. Moreover, the method cannot be used with very young birds, because of their sensitivity to surgery, and the insufficient development of the internal reproductive organs in such young birds.

A third method involves biochemical determinations of the concentrations of the steroid sex hormones, estradiol and testosterone, from blood and/or faecal matter. This procedure is based on the observation that male animals are characterized by a high concentration of testosterone in their blood, while females have high levels of estradiol. A disadvantage of this procedure is that it is technically very complicated and requires experienced

personnel for its operation. Another problem is that the procedure has no diagnostic value when used on young animals having low steroid hormone concentrations. Although the method can be applied to faecal matter rather than blood samples, it is often difficult to assign faecal samples to individuals birds when more than a few individual birds are under observation.

The fourth method, which is also an invasive method, is based on the occurrence of a specific W chromosome in female birds. Male birds have two Z sex chromosomes, "ZZ," whereas female birds have one W sex chromosome and one Z sex chromosome ("ZW"). In many, but not all bird species, the W chromosome has some unique staining characteristics and can, therefore, be observed and distinguished by microscopic examination of bird chromosome preparations. A disadvantage is that an expensive laboratory equipment is needed and highly qualified personnel are required for the performance of this procedure. As a further drawback associated with this method, in a number of bird species, including some parrots, an unambiguous identification of the W sex chromosome is not possible.

The fifth method available today, is a fingerprint based DNA analysis which involves the identification of sex specific DNA fragments by DNA

fingerprinting. According to this method, the DNA of individual birds is degraded with specific enzymes, resulting in the generation of specific fragments, which upon fractionation by electrophoresis forms a pattern specific for each individual bird, including its sex. The disadvantage of a fingerprint based DNA analysis is that it is technically very complicated and takes approximately one week to perform. Moreover, it requires isolation of very pure DNA, enzymatic digestion of the isolated DNA, fractionation by electrophoresis of the DNA fragments in the digest, and transfer of these fragments from the gel to a nylon membrane, followed by probing with a specific probe. Moreover, radioactive labelling of the probe is essential to the procedure, thus requiring the use of highly sophisticated equipment and facilities.

In view of the foregoing, it is evident that there is a demand for a simple, quick, and accurate method to determine the sex of birds, such as parrots.

Summary of the Invention

In brief, the present invention overcomes certain of the above-mentioned problems and shortcomings of the present state of the art through the discovery of novel nucleotide sequences derived from the W chromosome of a female parrot, the African

grey parrot, Psittacus erithacus. Uniquely, the novel nucleotide sequences of the present invention are highly female specific for this species of parrot. Moreover, they are believed to be highly female specific for almost all other species of parrot.

According to the present invention, the novel, universal nucleotide sequences are used as DNA or RNA probes in a quick and reliable procedure for sex determination of parrots. Unique to this procedure and as an advantage over prior invasive procedures utilized heretofore, the sex-typing procedures of the present invention can be completed within 24 hours. Moreover, the procedures of the present invention are reliable because virtually all species of parrots contain a sex specific component or components on the W sex chromosome which is closely similar to the sex specific component, i.e., the nucleotide sequences, isolated from the African grey parrot in accordance with the present invention.

In carrying out the present invention, it requires only a few microliters of blood, which can be collected easily without anaesthesia, from a wing vein or a toe of the parrot. As an alternative, the blood sample may be acquired from a blood feather such as a developing secondary or primary flight or tail feather.

To summarize a procedure of the present invention, a blood sample is first obtained from a parrot of choice, and DNA is then obtained from this blood sample (10 - 60 min). The DNA is denatured, bound to a nylon membrane and prehybridized (1-2 hrs). The DNA bound to the nylon membrane is than hybridized with a radioactive or nonradioactive DNA probe solution (12 hrs) of the present invention, and washed to remove the non-specifically bound DNA probe (1 hr). The specifically bound DNA probe is then visualized with the appropriate procedure (2 hrs) to determine the sex of the parrot.

The DNA or RNA probes used in the present invention are sex specific but not parrot-species specific. Radioactively or nonradioactively (biotin) tagged DNA or RNA probes of the present invention are successful in determining the sex of many parrot species in avian collections. The advantages of using the DNA or RNA probes, methods and kits of the present invention for the determination of the sex of parrots include, for example, 1) rapid determination (24 hrs); 2) major surgical procedures are not required; 3) the use of blood as a readily available source of DNA; 4) the use of a safe, stable, highly sensitive and highly sex specific but not species-specific DNA probe; 5) the use of simple procedures utilizing standard clinical and research

laboratory equipment which require minimal technical expertise for their operation; and 6) technical simplicity as compared to currently available procedures. Thus, the procedures of the present invention can be easily practiced by veterinarians and breeders in their offices and by other qualified personnel.

Accordingly, it should now be appreciated that the present invention is believed to provide a solution to the sex-typing parrot art that has long sought rapid and reliable methods for determining the sex of different species of parrots. This is accomplished by the present invention, as indicated above, through the identification of novel, universal nucleotide sequences which are useful as DNA or RNA probes that are complementary to DNA segments on the W chromosome which are characteristic for female parrots of the Psittacidae genus. Applications of the DNA or RNA probes contemplated by the present invention therefore include, for example, determination of the sex of parrots in captive breeding programs. Moreover, the applications of the probes include the determination of the sex of parrots in wild populations as part of, for example, research and ecological studies.

The above features and advantages will be better understood with reference to the FIGS.,

Detailed Description and Examples set out hereinbelow. It will also be understood that the biological materials, methods and kits of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the FIGS.

Reference is now made to the accompanying FIGS. in which are shown characteristics corresponding to the novel nucleotide sequences of the present invention from which certain of their features and advantages will be apparent:

FIG. 1 describes the nucleotide sequences of the two strands of the 461 bp sex specific DNA repeat of the African grey parrot. A randomly chosen second clone of the repeat shows 17 nts or 4% difference with the first clone.

FIG. 2 describes the structure of one of the strands of the 461 nt sex specific repeat of the African grey parrot in order to illustrate that it is made of a linear array of subfragments in which oligo dT fragments of 2-6 thymine (=T) residues are repeated at an average distance of 10.5 nts, which represents one complete turn of the DNA double helix.

FIG. 3 describes the signals obtained by hybridizing the cloned p^{32}-labelled African grey parrot sex specific DNA repeat element with three different amounts of nylon immobilized male and

female species: 1 = chattering lory (Lorius garulus); 2 = cherry-headed conure (Aratinga erythrogenys); 3 = sulphur-crested cockatoo (Cacatua galerita); 4 = blue-fronted amazon (Amazona aestiva); 5 = blue and gold macaw (Ara ararauna); 6 = African grey parrot (Pisttacus erithacus).

Detailed Description

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel nucleotide sequences, methods and kits.

The novel nucleotide sequences of this invention, preferably used as DNA or RNA probes, have been derived from the DNA of the female African grey parrot Psittacus erithacus. More particularly, they have been cloned as a fragment of 461 base pairs, obtained with the restriction nuclease MspI, in the vector pGEM3Z+ and the host Eschericia coli 31/17. The 461 base pair repeat is derived and isolated from a base pair repeat of about 570 base pairs and is made up of 43 subfragments in which oligo dT fragments of 2-6 thymine residues are repeated at an average distance of 10.5bp. More specifically, the 2-6 long oligo T fragments are part of 43 subfragments of a minimum length of about 4 nts and a maximum length of about 20 nts, and an average length

of about 10.5 nts which together form the 461 nt long fragment which is cloned. This latter fragment is again tandemly repeated 12,000 times. The DNA component is tandemly repeated with a copy number of approximately 12,000 copies per female African grey parrot genome and forms a substantial part of the W chromosomal DNA of this species. The nucleotide sequence of the component is conserved among the DNA of females of many other species of parrot. While the 461 base pair fragment has been sequenced as reported in FIG. 1, the remaining base pairs in the 570 base pair fragment from which the 461 base pair fragment has been derived and isolated have not been sequenced.

The novel procedure to demonstrate the presence of female W chromosome specific components in parrot DNA utilizes a probe comprising a trace amount of radio labelled female parrot DNA and a large excess (4000 fold) of unlabelled male DNA in an analysis of enzymatically digested and electrophoretically fractionated female parrot DNA. FOCUS (BRL) 14: 106-108 (1992). The excess unlabelled male DNA acts to dilute the radio-label in the components which are common to male and female DNA, so that common sequences do not produce any signal in the analysis, and the only signal observed is that produced by female specific components.

Utilizing this procedure with the restriction enzyme MspI, in a digest of the female African grey parrot, Psittacus erithacus, identification of a component of approximately 450 base pairs, which does not occur in the male DNA, is accomplished.

This fragment is isolated from the electrophoretically fractionated MspI digest and cloned in plasmid vector pGEMZ+ in the bacterium Eschericia coli 81/17. This cloned component has been used for the determination of the nucleotide sequence of the female specific component, its copy number and genomic organization, and the conservation among other species of parrots.

FIG. 1 shows that the sequence of the component has a molecular length of 461 base pairs. FIG. 2 shows that the fragment has an internal repeat structure in which groups of 2 - 6 thymine residues are repeated an average of 10.5 nucleotides. This sequence characteristic is typical for a rather unusual so called "curved DNA element".

A curved DNA is a double stranded (native) DNA which most often has short runs of 2-6 adenine or thymine residues at an average distance of 10.5 nts, which is just a complete winding of the DNA double helix. The curved nature of such DNA has been demonstrated by electronmicroscopy and circularization experiments. Curved DNA exhibits an

anomalous, slow electrophoretic velocity in polyacrylamide gels. This latter characteristic is usually taken as diagnostic evidence for the curved structure. The curved structure is an inherent property of such DNA and should be distinguished from so called bent DNA. The latter owes its curvature to interaction with certain proteins.

DNA components can occur either as unique or as repeated sequence elements. A quantitative analysis shows that the sex specific component amounts to approximately 0.3% of the genome of the female African grey parrot, whereas it amounts to at most 0.005% of the male genome. See Table I. Hence, the female African grey parrot genome contains about 12,000 copies of the sex specific DNA component of a length of 463 base pairs. These copies are tandemly organized in the genome. In situ hybridization to chromosomes of the African grey parrot shows strong hybridization of the DNA or RNA probes of the present invention to the W chromosome in the female whereas such hybridization is not observed in the male.

The DNA probes of the present invention can be produced by chemical synthesis, recombinant or cloning technology or any other methods available in the art so long as the methodology selected does not interfere with their utilities stated herein. Moreover, the DNA probes of the present invention may

be modified by adding, deleting and/or substituting nucleotides to form DNA probes of varying lengths which are functionally equivalent to the nucleotide sequences set forth in FIG. 1. In addition, RNA probes with nucleotide sequences contemplated by the present invention may be substituted for the DNA probes. Therefore, it is to be understood by those versed in this art that any DNA or RNA nucleotide sequence, including equivalents and active segments of the nucleotide sequences depicted in FIG. 1, which is complimentary to a DNA segment on a W chromosome which is characteristic for a female parrot of the Psittacidae genus is contemplated by the present invention. Examples of active nucleotide fragments in accordance with the present invention are those fragments derived by, for example, digesting the 461 base pair fragment with the enzyme SAU3A1 at a GATC site using digestion techniques known to those skilled in the art. When the 461 base pair fragment is digested with the enzyme SAU3A1, the 461 base pair fragment is cleaved at the GATC sites beginning with the nucleotide designated as 245 in the 5'-3' sequence and with the nucleotide designated as 213 in the 3'-5' sequence in FIG. 1 to generate four active fragments having, for example, bases corresponding to bases 1-248 and 249-461 in the 5'-3' sequence and

corresponding to bases 1-216 and 217-461 in the 3'-5' sequence as designated in FIG. 1.

The DNA or RNA probes of the present invention may be formed into kits which can be easily used by, for instance, veterinarians, breeders, as well as other qualified personnel interested in sex-typing parrots of the Psittacidae genus. A typical kit in accordance with this invention includes blood sample stabilizing Solution A; proteinase-K and sodium sarcosylate Solutions, or a commercially available fast DNA isolation kit; DNA denaturing and neutralizing Solutions; nylon membranes; biotin-labelled sex specific DNA or RNA probe; hybridization Solution B; wash Solutions C and D; and a detection system specific for the biotin tag so that all that is required to carry out the sex-typing methods of the present invention is standard laboratory equipment.

According to the present invention, blood is used as a source of DNA because avian erythrocytes are nucleated and contain DNA. Avian blood contains on average approximately 5 to 10 mgs of DNA per ml. In carrying out the sex typing of parrots in accordance with the present invention, blood samples, approximately 25 to 100 µl, are obtained by brachial (wing) vein puncture or by clipping of a toe nail or by removing a blood feather such as a secondary, or

primary flight feather or a tail feather. Clotting of the blood is prevented by immediate dilution, after collection, with an equal volume of a solution containing about 0.15M NaCl and about 0.05 M sodium EDTA, pH 7.5 (Solution A). In this solution, blood samples can be stored for at least a week in a refrigerator at 4°c.

The major contaminant of the DNA in avian blood is protein, which can be removed from the sample by protease digestion, e.g., phenol, or chloroform extraction in the presence of (cationic or anionic) detergents. [When chloroform extraction is used, the chloroform should be removed by for example centrifugation.] Accordingly, ~~10~~ µl of the 1:1 diluted blood sample (in Solution A ?) (containing on average 50 mgs of DNA) is diluted with 90 µl of Solution A and incubated for one hour at about 65°C with proteinase K (2 µgs per 100 µl) and sodium lauryl sarcosylate (0.2%). A commercially available DNA isolation system (e.g. from Invitrogen, 3985 Sorrento Valley Blvd, San Diego, CA 92121 or Washington Biotechnology Inc., 6917 Arlington Rd., Bethesda, MD 20814) can be used for this purpose.

DNA samples obtained are then denatured with NaOH (final concentration about 0.5 M) and neutralized with about 1 M NaH_2PO_4. Samples containing approximately 1 mg of denatured parrot DNA

are then loaded and immobilized onto nylon filters (0.2 µM pore size) with the aid of a slot or dot blot apparatus. The filters are then dried in vacuo at about 80°C for about two hrs to bind the DNA irreversibly to the filters.

Prior to hybridization with the probe, filters containing the DNA samples are prehybridized for about one hr at about 65°C in a solution containing about 0.9 M NaCl, 0.1 M Tris-HCl buffer pH 7.8, 0.05 M Na_2EDTA, 0.2% sodium lauryl sulphate and 500 µg per ml heparin as a blocking agent (Solution B).

The sex specific DNA fragment, obtained by cloning from the genome of the female African grey parrot as described above and recited in FIG. 1, is tagged by enzymatic or nonenzymatic procedures with radioactive P^{32} or nonradioactive biotin or fluorescent groups like fluoresceine or rhodamine using technologies available to these in the labeling art. It should be understood that the DNA probe may also be tagged with fluorescent dyes, such as fluoreseine or rhodamine using techniques known to those versed in this art. In the present example the DNA probe is tagged by, e.g., labelling with biodUTP using nick translation or random primer extension. As to an RNA probe, it is labelled with, e.g., bioUTP using T7 RNA polymerase (pGEM3Z+ has a T7 RNA

polymerase promoter site). The tagged, denatured DNA probe is then incubated for approximately 60 - 65°C and at a concentration of about 25 ng per ml of Solution B with the nylon filters containing the parrot DNA. Subsequent to incubation, the filters are washed first at room temperature with 1 x SSC (= 0.15 M NaCl, 0.015 M sodium citrate pH. 7.0, Solution C) (five times for about five minutes with about 100 ml) and subsequently one time for about two minutes at about 55°C with approximately 100 ml 1 x SSC containing about 0.1% sodium lauryl sulphate (Solution D). Binding of radioactively labelled probe is visualized and quantified by autoradiography and/or a betascope. In this Example, bound probes labelled nonradioactively with biotin are visualized through a commercially available detection process which involves a specific color reaction or chemiluminescence. The specific colour reaction involves, e.g., the binding of a streptavidin or avidin phosphatase conjugate to the biotin residue (streptavidine and avidine are proteins which have a ver yhigh specific affinity for biotein; phosphatase as an enzyme which hydrolyes phosphate esters.) and the subsequent hydrolysis of a so-called chromogenic substrate which consists of an uncoloured phosphate ester which becomes strongly coloured after removal of the phosphate by the phosphatase. Examples of

such esters are bromo-indoxyl phosphate which is uncoloured, but which generates dark blue indigo after removal of the phosphate. Chemiluminescent detection systems make use of chemiluminescent substrates in a similar phosphatase catalyzed reaction, followed by photographic detection of the emitted light.

The present invention will now be further illustrated with reference to the following Examples.

Example I

SEX SPECIFIC DNA FRAGMENT

Female african grey parrot DNA (10 ug) is digested in 100 µl of a buffer (containing 6 mM MgCl2, 6 mM Tris-HCl buffer pH 7.5, 50 mM NaCl) with 20 units of the restriction endonuclease Mspl for 3 hrs at 37° C. For identification purposes of the sex specific DNA fragment, 10 µl of the digest is fractionnated by electrophoresis through a 1% agarose gel in TPE buffer (TPE = 0.08 M Tris-phosphate pH 7.5, 0.008 M EDTA). A southern blot of the gel is made and probed with a genomic DNA probe made from 25 ng radioactively (P^{32}) labelled female DNA [labelled by random primer extension, Feinberg, A.P. et al.: Anal. Biochem., 132:6-13 (1983), preannealed to 100 µg reiterated, denatured male African grey parrot Cot2 DNA (Cot indicates a degree of repetitivity of the DNA sample, see: De Kloet pH and de Kloet SR

(1992) Molecular determination of the sex of parrots (FOCUS (BRL) 14:106-108 (1992)).

After identification of the sex specific DNA fragment by autoradiography or with a betascope, a preparative (larger) gel is run and the gel slice containing the 461 bp sex specific fragment cut out. The sex specific fragment is isolated from the gel by the powdered glass procedure, Vogelstein, B. et al.: Proc. Natl. Acad. Sci. USA, 76:615-619 (1979). The fragment is ligated into the powdered glass procedure. See Vogelstein B. et al.: Proc. Natl. Aca. Sci. USA, 76:615-619 (1979). The fragment is ligated into the Acc1 site of the plasmid pGEM3Z+ and the ligation product is used to transform E. coli 71/18 using the $CaCl_2$ procedure (Maniatis, T. et al. (1982) "Molecular cloning; a laboratory manual." Cold Spring Harbour Laboratory. Cold Spring Harbour, NY, 2) probe labelling with biotin:). The transformed bacteria are spread on 20 ml agar plates containing Luria bertani (LB) medium (LB = 1% bacto=tryptone, 1% NaCl, 0.5% bacto yeast extract pH 7.4) containing per ml 50 µgs of penicillin, 150 µgs bromo-idnolyl-beta-galactoside and 75 µg isopropyl beta thiogalactoside. After growth vernight at 37°C, the white colonies (containing plasmids with inserts) are selected and transferred to fresh FL plates covered with a Nylon filter. Control plates without

a filter are also inocculated with the colonies in the same pattern. After growth until the colonies are detectable, the nylon filter is removed from the plate and dried in a vacuum oven at 80°C. Colonies with a sex specific insert are identified by colony hybridization (Maniatis, T. et al. (1982) "Molecular cloning; a laboratory manual." Cold Spring Harbour Laboratory. Cold Spring Harbour, NY, 2) probe labelling with biotin:) using the same genomic sex specific DNA probe as is used initially for determination of the sex of parrots (FOCUS (BRL) 14:106-108 (1992)). Positive colonies are identified by autoradiography or a betascope, and identified on the control plates which are incubated without a nylon filter because of the similarity in pattern of application which is used. After identification the colonies are transferred into LB medium without agar, containing 50 µg penicillin per ml, and incubated overnight at 37°C. The bacteria are collected by centrifugation and resuspended in LB + ampicillin containing 15% glycerol and stored at -80°C.

Example II

DETERMINATION OF THE SEX OF PARROTS WITH THE AFRICAN GREY PARROT SEX SPECIFIC DNA COMPONENT

In carrying out a procedure in accordance with the present invention to determine the sex of a parrot, twenty-five (25) µl blood is removed from a wing vein or toe with a heparinized capillary and

suspended in 0.5 ml of buffer A (a solution containing 0.05 M Na_2EDTA and 0.15 M NaCl (pH 7.4)). Of this suspension, 100 µl (corresponding to 5 µl of blood) is taken and diluted with 200 µl of a solution containing 8% sodium dodecyl trimethylammonium bromide, 1.5 M NaCl, 100 mM Tris-HCl buffer pH 8.6, 50 mM EDTA. After heating for two minutes at 68°C, vortexing for two minutes with 300 µl of chloroform and centrifugation for two minutes in an Eppendorf centrifuge, the aqueous phase (top layer) (250 µl) is collected. Since avian blood contains approximately 5 µg DNA per µl, this aqueous phase contains approximately 25 (5 x 5) µg of DNA per 250 µl, or 1 µg of DNA per 10 µl. Fifty µl of the aqueous phase is then taken (containing approximately 5 µg of DNA), diluted with 50 µl to TE (0.01 M Tris-HCl buffer pH 8.0, 0.001 M Na_2EDTA), and the DNA is denatured (make single stranded) by the addition of 100 µl of 1 M NaOH. After five (5) minutes, the denatured DNA is neutralized with 200 µl 1 M NaH_2PO_4 and 100 µl of 1.5 M NaCl is added to a final volume of 500 µl. 100 µl of this solution (containing approximately 1 µg of DNA) is applied to a Nylon filter with the aid of a slot blot or dot blot apparatus. The filter is dried and baked in an oven at 80°C. For hybridization, the filter with the bound denatured DNA is then preincubated for thirty minutes at 60°C with 0.2 ml

per cm^2 of a solution containing 0.9 M NaCl, 0.002 M Na EDTA, 0.02 M Tris-HCL pH 8.0, 0.1% sodium dodecyl sulphate and 500 µg per ml of heparin (as the blocking agent to prevent direct binding of the probe to the filter without binding to the DNA) and 1000 µg per ml of sodium pyrrophosphate. Heat denatured radioactively or nonradioactively (biotin) labelled parrot sex specific probe (40 - 100 ng per 10 ml buffer) is then added and the incubation continued overnight. Subsequently, the filters are washed with 1 x SSC to remove the unhybridized probe, and the bound radioactive label visualized by autoradiography or a betascope. The biotin labelled probe is visualized with any of the appropriate techniques like streptavidine - alkaline phosphatase etc.

In the event the blood sample is taken from a blood feather, the procedure is as follows. A growing primary or secondary feather is collected and the featherpulp is squeezed out of the feather into 1 or 2 ml (depending on the size of the feather) of buffer A (see above) or any other stabilizing solution (Dulbecco's modified Eagles etc.). To this suspension is added 75-150 µl of a solution of 10 µg per ml collagenase (Closteridium haemolyticum) in water and the mixture incubated for two hours at 37°C. After gentle homogenization DNA is prepared from the resulting cells as described above for blood

DNA. After measuring the amount of prepared DNA with a spectrophotometer, the DNA is again alkali denatured, applied to a nylon filter and the filter prehybridized and hybridized with a probe as described above.

It should be understood that the procedure described is used to generate the results in Example III and as depicted in FIG. 3. It should also be understood that the procedures described herein are used to sexually type the parrots listed in Table I.

TABLE I

Species	Female	male
Blackmasked lovebird	8.3	0.3
King parrot	40.2	2.2
Bluefronted amazone	7.6	0.7
Double yellow headed amazone	8.4	0.7
Aymara parakeet	15.8	1.5
Crimsonwinged parrot	16.4	1.3
Blue and gold macaw	12.4	1.2
Scarlet macaw	14.7	0.9
Severes macaw	13.8	1.4
Redfronted conure	15.8	1.3
Sun conure	17.2	1.7
Goffins cockatoo	30.3	0.9
Moluccan cockatoo	35.0	1.3
Sulphur-creasted cockatoo	33.7	1.1
Gang-gang cockatoo	30.2	0.8
White-tailed black cockatoo	38.3	0.7
Red-tailed black cockatoo	35.3	0.9
Rosebreasted cockatoo	31.3	1.1
Chattering lory	13.5	1.3
Budgerigar	23.5	0.7
Cockatiel	34.5	0.6
Blue-headed pionus	12.4	0.9
Eastern rosella	25.6	0.4
Princess of Wales parakeet	24.3	0.5
Palm cockatoo	12.7	0.7
Alexandrine parakeet	20.6	1.3
Ring-necked parakeet	30.3	2.1
African grey parrot	100.0	2.5
Pesquet's parrot	8.5	0.3

Table I. Use of PARsex1 for molecular sex determination of selected psittacines. Values represent the average amount of PARSEX1 present in DNA of 2 - 5 males and females of the species indicated, calculated as percentage of the amount present in the female African grey parrot. PARsex1 refers to the DNA probe having the sequence shown in FIG. 1.

Example III

RESULTS

FIG. 3 shows that in a blotting experiment, DNA of the African grey parrot as well as of other species of parrots, belonging to the different major subfamilies of the Psittacidae, namely, the conure, the cockatoo, the lory, the amazone and the macaw also produce a sex specific signal with the radioactively labelled female specific DNA component of the African grey parrot, showing that the sex specific characteristics of the component is conserved among the parrot family. In FIG. 3, different amounts of DNA of males and females of the species indicated are immobilized on a nylon filter and hydribized with the p^{32}-labelled African grey parrot sex specific DNA repeat element as the probe. The sex specific hybridization of this probe with DNA of all six species shows that the sex specific DNA component is structurally conserved between the different species and can be used for the determination of all species.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: de Kloet, Siwo R.

(ii) TITLE OF INVENTION: Sex-Specific DNA Probe For Parrots, Methods And Kits

(iii) NUMBER OF SEQUENCES: 44

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ruden, Barnett, McClosky, Smith, Schuster & Russell, P.A.
(B) STREET: 200 East Broward Boulevard
(C) CITY: Fort Lauderdale
(D) STATE: FL
(E) COUNTRY: USA
(F) ZIP: 33301

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Manso, Peter J.
(B) REGISTRATION NUMBER: 32,264
(C) REFERENCE/DOCKET NUMBER: FL20979-34

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 305-527-2498
(B) TELEFAX: 305-764-4996

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 460 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA ACCAATTTTA 6

AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT GAACAAGCTT TAAGTCCTTT 12

TTGGTTGCTT GTAAATGATT TTTGGAGTTT TCTAACCCCT TTTGAGCCAT TTTTTCTGTT 180

TCTAACCCAT TTTTTCAACA GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC 240

CATTGATCCC ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC 300

CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT AGCCCATTCC 360

TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA TTTTTGGTCT TGTCAAATGC 420

ATTTTTCACC TCTTCTGACT CGCTTGAAGA CCTTTTTGCC 460

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTTTCGTCC 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATTCCTAACC AC 12

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATTAAAGCCT 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATTTTTCACC C 11

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTCCAACC A 11

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTTTAAGCA 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATTTGTGGTC 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTTCAAACA CA 12

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTTTTCACCA 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTTGAACAA G 11

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTTTAAGTC 9

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTTTTTGGTT G 11

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGTAAATG 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATTTTTGGA 9

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTTTTCTAAC CC 12

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTTTTGAGCC 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTTTTTCT 9

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTTTCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTTTTTCAA CA 12

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTCTAGCTC G 11

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTTTAAGTA 9

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTTTTG 7

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTTTTTTCTA ACCC 14

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATTGATCCCA TGACTAATCA 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTTTTAAGCC 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTTTTTGTCC 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATTTCTGACC CAT 13

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTTTGCCCAG CTCTAG 16

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CTTT 4

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTTTAAGCC 9

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTTTTTCTCC 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ATTTCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTTTCTAGCC C 11

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATTCCTGACC T 11

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTTTTAAGCC T 11

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATTCCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATTTCCAACC C 11

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ATTTTTGGT 9

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTTGTCAAAT GC 12

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

ATTTTTCACC T 11

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTCTGACTC G 11

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTTGAAGAC 9

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTTTTTGCCG 10

The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the spirit and scope of the appended claims are intended to be embraced herein.

Having described my invention, I claim:

CLAIMS:

(1) An oligonucleotide probe having an effective number of nucleotides for hybridizing specifically with a DNA segment which is characteristic for female parrots of the Psittacidae genus.

(2) An oligonucleotide probe as recited in claim 1, the DNA segment being located on the W chromosome of a female parrot of the Psittacidae genus.

(3) An oligonucleotide probe as recited in claim 2, said probe further including a labeled moiety so that, when said labeled probe is hybridized to the DNA segment, the DNA segment on the W chromosome can be detected.

(4) An oligonucleotide probe as recited in claim 3, said labeled moiety being a fluorophore.

(5) An oligonucleotide probe as recited in claim 4, said fluorophore being selected from a group consisting of a fluorescein, a rhodamine or any other fluorophore.

(6) An oligonucleotide probe as recited in claim 3, said labeled moiety being selected from a group consisting of biotin and a radioisotope.

(7) An oligonucleotide probe as recited in claim 2, said probe having a periodicity of groups of about 2 to about 6 thymine or adenine residues, said groups being repeated on average of about 10.5 nucleotides.

(8) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe further having a curved DNA element.

(9) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe having a nucleotide sequence selected from a group having the following 5′-3′ sequence or 3′-5′ sequence or an equivalent sequence or an effective segment thereof:

```
5′-GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA
3′-CAAAAGCAGG TAAGGATTGG TGTAATTTCG GATAAAAAGT GGGTAAAGGT

ACCAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
TGGTTAAAAT TCGTTAAACA CCTGTAAAGT TTGTGTCAAA AGTGGTGAAA

GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
CTTGTTCGAA ATTCAGGAAA AACCAAGCAA CATTTACTAA AAACCTCAAA

TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
AGATTGGGGA AAACTCGGTA AAAAAGACAA AGATTGGGTA AAAAAGTTGT

GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
CAAGATCGAG CCAAATTCAT CAAAAACGAA AAAAGATTGG GTAACTAGGG

ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC
TACTGATTAG TCAAAATTCG GCAAAAACAG GTAAAGACTG GGTAGAAACG

CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
GGTCGAGATC GAAACAAATT CGGCAAAAAG AGGTAAAGAT TGGGCAAAGA

AGCCCATTCC TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA
TCGGGTAAGG ACTGGACAAA ATTCGGATAA GGATTGGGTA AAGGTTGGGT

TTTTTGGTCT TGTCAAATGC ATTTTTCACC TCTTCTGACT CGCTTGAAGA
AAAAACCAGA ACAGTTTACG TAAAAAGTGG AGAAGACTGA GCGAACTTCT

CCTTTTTGCC G-3′
GGAAAAACGG C-5′
```

(10) An oligonucleotide probe as recited in claim 1, said probe being a DNA probe.

(11) An oligonucleotide probe as recited in claim 1, said probe being a RNA probe.

(12) A method of determining the sex of a parrot of the Psittacidae genus, said method comprising:

hybridizing the labeled probe of claim 3 to the female chromosome DNA of the animal; and

detecting the labeled probe hybridized to the DNA segment on the female chromozome of the parrot to identify the female chromosome of the parrot.

(13) A method as recited in claim 12, said detecting step comprises:

visualizing the labeled probe hybridized to the DNA segment.

(14) A method as recited in claim 12, the labeled probe being a labeled DNA probe.

(15) A method as recited in claim 12, the labeled probe being a labeled RNA probe.

(16) A kit for determining the sex of a parrot of the Psittacidae genus, said kit comprising:

a.) an oligonucleotide probe of claim 1; and

b.) components for carrying out an assay to determine the sex of the parrot.

(17) A kit as recited in claim 14, said oligonucleotide probe being a DNA probe.

(18) A kit as recited in claim 14, said oligonucleotide probe being a RNA probe.

FIG. 1. The nucleotide sequence of the sex specific DNA fragment of the African grey parrot.

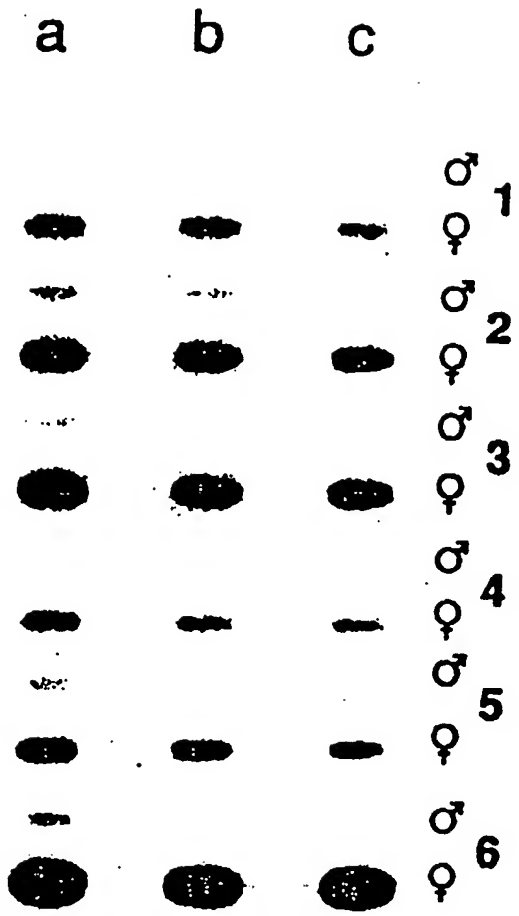
SEQ ID NO:1:

```
5'-GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA
3'-CAAAAGCAGG TAAGGATTGG TGTAATTTCG GATAAAAAGT GGGTAAAGGT

   ACCAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
   TGGTTAAAAT TCGTTAAACA CCTGTAAAGT TTGTGTCAAA AGTGGTGAAA

   GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
   CTTGTTCGAA ATTCAGGAAA AACCAAGCAA CATTTACTAA AAACCTCAAA

   TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
   AGATTGGGGA AAACTCGGTA AAAAAGACAA AGATTGGGTA AAAAAGTTGT

   GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
   CAAGATCGAG CCAAATTCAT CAAAAACGAA AAAAGATTGG GTAACTAGGG

   ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC
   TACTGATTAG TCAAAATTCG GCAAAAACAG GTAAAGACTG GGTAGAAACG

   CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
   GGTCGAGATC GAAACAAATT CGGCAAAAAG AGGTAAAGAT TGGGCAAAGA

   AGCCCATTCC TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA
   TCGGGTAAGG ACTGGACAAA ATTCGGATAA GGATTGGGTA AAGGTTGGGT

   TTTTTGGTCT TGTCAAATGC ATTTTTCACC TCTTCTGACT CGCTTGAAGA
   AAAAACCAGA ACAGTTTACG TAAAAAGTGG AGAAGACTGA GCGAACTTCT

   CCTTTTTGCC G-3'
   GGAAAAACGG C-5'
```

FIG. 2. Internal repeat structure of the 461 nt sex specific repeat of the African grey parrot. The subfragments begin with the nucleotide preceeding the individual oligo dT_{2-6} segments. Initial nt indicates the position of the initial nt in FIG. 1.

Fragment nr	Initial nt		fragment sequence	fragment length	oligo dT length
1	1	SEQ ID NO:2:	5'- GTTTTCGTCC	10	4
2	11	SEQ ID NO:3:	ATTCCTAACCAC	12	2
3	23	SEQ ID NO:4:	ATTAAAGCCT	10	2
4	33	SEQ ID NO:5:	ATTTTTCACCC	11	5
5	44	SEQ ID NO:6:	ATTTCCAACCA	11	3
6	55	SEQ ID NO:7:	ATTTTAAGCA	10	4
7	65	SEQ ID NO:8:	ATTTGTGGTC	10	3
8	75	SEQ ID NO:9:	ATTTCAAACACA	12	3
9	87	SEQ ID NO:10:	GTTTTCACCA	10	4
10	97	SEQ ID NO:11:	CTTTGAACAAG	11	3
11	108	SEQ ID NO:12:	CTTTAAGTC	9	3
12	117	SEQ ID NO:13:	CTTTTTGGTTG	11	5
13	128	SEQ ID NO:14:	CTTGTAAATG	10	2
14	138	SEQ ID NO:15:	ATTTTTGGA	9	5
15	147	SEQ ID NO:16:	GTTTTCTAACCC	12	4
16	159	SEQ ID NO:17:	CTTTTGAGCC	10	4
17	169	SEQ ID NO:18:	ATTTTTTCT	9	6
18	178	SEQ ID NO:19:	GTTTCTAACCC	11	3
19	189	SEQ ID NO:20:	ATTTTTTCAACA	12	6
20	201	SEQ ID NO:21:	GTTCTAGCTCG	11	2
21	212	SEQ ID NO:22:	GTTTAAGTA	9	3
22	221	SEQ ID NO:23:	GTTTTTG	7	5
23	228	SEQ ID NO:24:	CTTTTTTCTAACCC	14	6
24	242	SEQ ID NO:25:	ATTGATCCCATGACTAATCA	20	2
25	262	SEQ ID NO:26:	GTTTTAAGCC	10	4
26	272	SEQ ID NO:27:	GTTTTTGTCC	10	5
27	282	SEQ ID NO:28:	ATTTCTGACCCAT	13	3
28	295	SEQ ID NO:29:	CTTTGCCCAGCTCTAG	16	3
29	311	SEQ ID NO:30:	CTTT	4	3
30	315	SEQ ID NO:31:	GTTTAAGCC	9	3
31	324	SEQ ID NO:32:	GTTTTTCTCC	10	5
32	334	SEQ ID NO:33:	ATTTCTAACCC	11	3
33	345	SEQ ID NO:34:	GTTTCTAGCCC	11	3
34	356	SEQ ID NO:35:	ATTCCTGACCT	11	2
35	367	SEQ ID NO:36:	GTTTTAAGCCT	11	4
36	378	SEQ ID NO:37:	ATTCCTAACCC	11	2
37	389	SEQ ID NO:38:	ATTTCCAACCC	11	3
38	400	SEQ ID NO:39:	ATTTTTGGT	9	5
39	409	SEQ ID NO:40:	CTTGTCAAATGC	12	2
40	421	SEQ ID NO:41:	ATTTTTCACCT	11	5
41	432	SEQ ID NO:42:	CTTCTGACTCG	11	2
42	443	SEQ ID NO:43:	CTTGAAGAC	9	2
43	452	SEQ ID NO:44:	CTTTTTGCCG -3'	10	5

FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08023

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07H 21/04; C12Q 1/68
US CL :536/24.1; 435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/24.1; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Genomics, Volume 14, No. 2, issued 1992, C. S. Madsen et al., "Highly repeated DNA sequences in birds: The structure and evolution of an abundant, tandemly repeated 190-bp DNA fragment in parrots", pages 462-469, see entire document.	1-11 ------- 12-18
Y,P	EXS, Volume 67, issued October 1993, J. Mathe et al., "Paternity testing of endangered species of birds by DNA fingerprinting with non-radioactive labelled oligonucleotide probes", pages 387-393, see entire document.	1-18
Y,P	Genome, Volume 37, No. 3, issued 1994, "Sequence conservation of an avian centromeric repeated DNA component", pages 351-355, see entire document.	1-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 OCTOBER 1994	Date of mailing of the international search report 20 OCT 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LISA ARTHUR Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08023

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Poultry Science, Volume 72, No. 5, issued 1993, J. L. Halverson et al., "Genetic control of Sex determination in birds and the potential for its manipulation", pages 890-896, see especially pages 893-895.	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08023

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE, DERWENT BIOTECHNOLOGY ABSTRACTS, CURRENT BIOTECH ABS, DERWENT WORLD PATENTS, PASCAL, UEMBL, GENBANK
search terms: parrots, psittacidae, oligonucleotide, probe, W chromosome, tandem repeat